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Research Article

Development and Validation of Stability Indicating UV Spectrophotometric Method for the Estimation of Fosfomycin in Bulk and Tablet Dosage Form

Dhananjay M Patil*, Sapana S Chaudhary, Vinod A Bairagi and Rakesh M Bachhav

Department of Pharmaceutics, K.B.H.S.S. Trust's Institute of Pharmacy, Malegaon-Camp, Malegaon, Maharashtra, India

***Corresponding Author:** Dhananjay M Patil, Department of Pharmaceutics, K.B.H.S.S. Trust's Institute of Pharmacy, Malegaon-Camp, Malegaon, Maharashtra, India.

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Abstract

A simple, reproducible, robust and precise as well as cost effective stability indicating UV spectrophotometric method has been developed of Fosfomycin in bulk and pharmaceutical formulations. The maximum UV spectrum wavelength is 254 nm by scanned in the range of 200 to 400 nm of Fosfomycin stock solution. The obeyed Beer's law in the concentration range of 05 - 25 µg/ml. The Fosfomycin subjected to forced degradation studies includes are the extremely unfavorable environmental and chemical conditions, were giving to the ICH guideline. And the results were found to be acceptable in range and application of this method was effectively in pharmaceutical dosage form without any interfering by the additive.

Keywords: Fosfomycin; Forced Degradation; UV Method; Validation; ICH Guidelines

Introduction

During the pharmaceutical development of a new drug, it is necessary to select as soon as possible the formulation with the best stability characteristics.

Regulations regarding stability testing for registration application are provided by current International Commission for Harmonization (ICH), which emphasizes the stress testing conditions with the aim of assessing the effect of severe conditions on the drug in practice, the effects of pH and temperature changes on drug stability are often used in such studies.

The results of force degradation are useful in the estimation of a drug product shelf life during pharmaceutical development and its storage [1].

Fosfomycin, a new therapeutic drug chemically known as (2R,3S)-3methyloxiran - 2yl - 2 - phosphonic acid was used to treat urinary tract infection [1]. Figure 1 shows that structure of Fosfomycin.

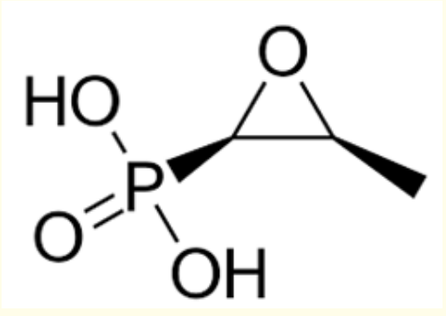

Figure 1: Chemical structure of Fosfomycin

According to literature survey, the developed the HPLC-UV [3,4] and LC–MS [2] methods for estimation of Fosfomycin in bulk drug. The Capillary Electrophoresis method reported by [5], for the Determination of fosfomycin in biological fluids by capillary electrophoresis. Recently [6], Multi-spectroscopic investigation of the binding interaction of fosfomycin with bovine serum albumin was done.

So far, determination of Fosfomycin in pharmaceutical formulations, there is no UV assay methods developed in the literature. Amongst the several methods existing for the determination of drugs, spectrophotometry continues to be very popular, because of their simplicity, specificity and low cost.

In the present a guileless, commercial, sensitive, fast, and precise analytical method for the routine analytical method with enhanced detection range for estimation of Fosfomycin in pure form and in its pharmaceutical dosage forms was developed and validated. The method was also tested for forced degradation studies, according to the ICH guidelines which can be used for the routine analysis of Fosfomycin in pure form and formulations.

Materials and Methods

Fosfomycin was obtained as a gift sample from Cipla Pvt. Ltd. Mumbai. A Lab India UV/VIS double beam spectrophotometer (model 3000 +) was used for all analytical purpose. Chemicals and solvent were used as analytical grade reagent purchased from Research lab, Mumbai.

Preparation of standard stock solution

Weighed accurately 10 mg of Fosfomycin and transferred in 100 ml volumetric flask containing 20 ml of distilled water the remaining volume makeup with distilled water to give 100 µg/ml stock solution.

Preparation of calibration curve for Fosfomycin

Scanning the wavelength in the range of 200 - 400 nm with stock solution for λ max determination of drug by using UV-VIS spectrophotometer show in figure 2. The remaining standard solution of Fosfomycin were prepared in 10 ml volumetric flask by pipetted out (1, 2, …. 8 ml) stock solution and the volume was make up with double distilled water. The absorbance was measured at λ max against reagent as a blank. The plotting the calibration curve with absorbance v/s concentration (µg/ml) by measuring the correlation coefficient. The calibration curve data and analytical parameters are presented in table 1 and table 2 respectively.

Sr. No.	Conc. (µg/ml)	Absorbance
1	5	0.060
2	10	0.118
3	15	0.172
4	20	0.209
5	25	0.274

Table 1: Calibration curve data for Fosfomycin.

Parameters	Result
Measured wavelength (λ_{max})	254 nm
Beers law limit (µg/ml)	5 - 25 ppm
Regression equation (y = m x + c)	Y = 0.0052x + 0.0011
Slope	0.005
Intercept	0.003
Correlation coefficient (r)	0.9992
LOD µg/ml	0.19
LOD µg/ml	0.83

Table 2: Optical characteristics of the proposed method.

Estimation of Fosfomycin in sachet

Five Fosirol® sachets contents were mixed and weighed and to measure the average weight of one sachet. Take the 100 ml volumetric flask with containing 30 ml double distilled water and weigh the equivalent quantity of 26.66 mg of fosfomycin powder transferred in it. The volumetric flask was subjected to the sonicated for 15 minutes. and volume was made up to mark with the same solvent and filter through Whatman filter paper no. 42. Make the final concentration (20 µg/ml) from the above stock solution by using distilled water. Then the measured the absorbance at 254 nm.

Method Validation

The method was validated according to ICH Q2B guidelines to determine the Linearity, sensitivity, precision, and accuracy of the analyte [7-9]. The calibration curve was obtained in a concentration range from 5 - 25 µg/ml for fosfomycin. The response of the drug was found to be linear in the investigation concentration range and performing least square regression analysis and the accuracy of the projected method was checked using standard addition method and recovery studies were carried out at 80%, 100% and 120% of target concentration [10]. The percent analytical recovery was calculated by comparing the concentration resulted with the addition of spiked samples with actual expected theoretical increase in concentration. Inter-day and intra-day precision were determined by performing analysis on two consecutive days and at two different time intervals in a day. LOD and LOQ of the planned methods were calculated [11]. Recovery of the analyte of interest from a given matrix can be used as a measure of the accuracy or the bias of the method [12].

Stability Studies of Fosfomycin

Stability studies were performed by forced degradation study of Fosfomycin and it includes the study of effect of oxidation, photolysis, temperature and susceptibility to hydrolysis across a widespread range of pH values.

For oxidation study 0.1%, 1% and 3% H_2O_2, for basic hydrolysis 0.1, 1 N NaOH, for acidic hydrolysis 0.1, 1.0 N HCl was used. The photolysis studies of drug were treated with sunlight for 3 days and thermal stress study by heating the drug at 60°C for 2 hrs.

Results and Discussion

The development of a guileless, commercial, sensitive, fast, and precise analytical method for the routine quantitative determination of Fosfomycin by reduce the unnecessary sample preparations and the effect on labor and materials cost. The absorption spectrum of Fosfomycin in double distilled water is shown in figure 2.

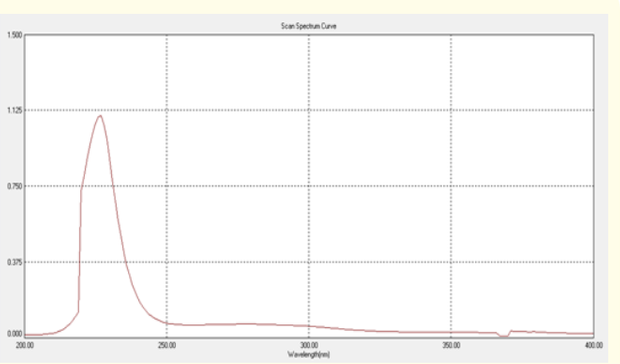

Figure 2: UV spectrum of Fosfomycin.

The Fosfomycin λ max was determined (254 nm) by scanning the drug sample solution in the UV region. Calibration curve data of Fosfomycin was constructed in the concentrations range of 5 - 25 µg/ml. and obeys the Beer's law in this concentration range (Figure 3).

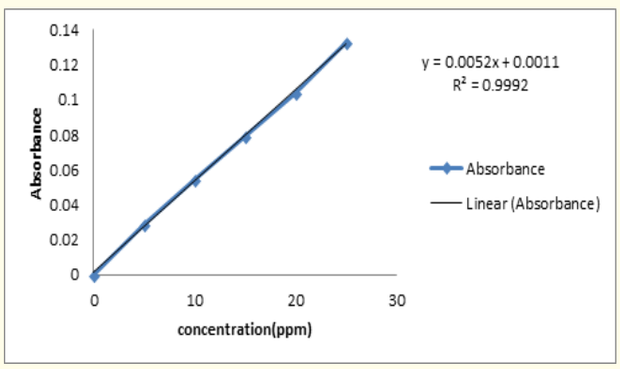

Figure 3: Calibration curve of Fosfomycin at 254 nm.

The regression equation of Fosfomycin was found to be Y = 0.0052 x + 0.0011 and the correlation coefficient (r) was found to be 0.9992. The characteristic of the calibration plot is presented in table 1. To performing the repeated analysis of standard solution was used to assess the accuracy and precision of the proposed methods (Table 3 and 4). The LOD and LOQ were found to be 0.19 µg/ml and 0.83 µg/ml respectively.

Level of % recovery	% Mean* recovery	S.D.	R.S. D	SE
	Fosfomycin			
80	100.10	0.610	0.617	0.338
100	100.11	0.510	0.517	0.301
120	100.20	0.475	0.465	0.291

Table 3: Result of recovery studies.

* Mean of three determinations at each.

Parameters	Concentrations (µg/ml)		
	05	15	25
Intraday*			
% mean ± S. D	2.06 ± 0.208	50.22 ± 0.602	79.77 ± 0.769
%RSD	0.995	0.012	0.991
SE	0.0352	0.04057	0.04376
Interday*			
% mean ± S. D	20.08 ± 0.32	50.77 ± 0.16	80.47 ± 0.119
%RSD	0.451	0.320	0.128
SE	0.02997	0.04565	0.04241

Table 4: Statistical validation for interlay and intraday precision.

*Denotes average of three determinations, SE - Standard error.

To study the correctness of the planned method and to check the interfering from excipients used in dosage forms, recovery experiments were carried out by the standard addition method. The mean recovery was found to be 100.10-100.20. The planned methods can be effectively applied for assay in tablet dosage forms without any interference (Table 3).

The selected concentration within the calibration range was prepared in double distilled water and analyzed with the relevant calibration curves to determine the intra- and inter-day variability.

To determine the precision of the method Fosfomycin solutions at concentration 05, 15, 25 µg/ml were analyzed each in triplicate. Solutions for the standard curves were prepared fresh every day. The method was found to be precise. The % RSD values for interday precision at concentration 05, 15, 25 µg/ml was found to be 0.995, 0.012, 0.991 respectively and for intraday precision it was 0.451, 0.320, 0.128 respectively. Results are shown in table 4.

The application of this procedure is explained in the experimental section. The obtained results demonstrate the validity and accuracy of the proposed method for the determination of Fosfomycin in sachets. The stability studies indicate that appreciable changes were observed by treating the drug with sun light, thermal stress, oxidation, acid and basic hydrolysis, however there was appreciable change with all these stress conditions. The results are shown in table 5.

Sr. No.	Conditions applied	Conc. taken (µg/ml)	Average Conc. Found (µg/ml)	Observation
1	Acidic hydrolysis (0.1, 1N HCl)	20 µg/ml	24.37 µg/ml	Degraded
2	Basic hydrolysis (0.1, 1N NaOH)	20 µg/ml	23.33 µg/ml	Degraded
3	H_2O_2 (0.1, 1, 3%)	20 µg/ml	Change in λ_{max}	Degraded
4	Thermal stress (60^0C, 2 hrs)	20 µg/ml	Change in λ_{max}	Degraded
5	Sunlight treatment (1, 2, 3 day)	20 µg/ml	28.35 µg/ml	Degraded

Table 5: Result of forced degradation study of Fosfomycin.

Conclusion

These results reveal that the developed method was guileless, commercial, sensitive, fast, and precise analytical method for the routine quantitative determination of Fosfomycin tablet in pharmaceuticals without any interference from the excipients. Based on forced degradation studies according to the ICH guidelines, this method can be used for the routine and quality control analysis of Fosfomycin in raw material and pharmaceutical formulations.

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